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Direct Development in the Sea Urchin *Phyllacanthus parvispinus* (Cidaroidea): Phylogenetic History and Functional Modification

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Abstract. Development in the Australian sea urchin *Phyllacanthus parvispinus* (Echinoidea: Cidaroidea) is of interest because it has a highly modified, lecithotrophic larva, and because it belongs to an echinoid group whose development has been little studied. This study documents early development and metamorphosis in *P. parvispinus* and considers the evolution of features unusual in echinoid ontogeny. Some features, such as lack of a vestibule, occur in other cidaroids, and are likely a product of ancestry. Other unusual features, such as larger gametes, an equal fourth cleavage, a wrinkled blastula, and accelerated development of the adult rudiment, are characteristic of other direct developing echinoids, and are probably functional modifications for altered developmental mode. Since the Cidaroidea form the sister group to the more derived Euechinoidea, cidaroid development is critical in assessing the phylogeny of ontogeny among echinoids. The distribution of developmental features among extant echinoids suggests that the extinct ancestor of cidaroids and euechinoids had planktotrophic larvae that lacked a vestibule during formation of the juvenile rudiment.

Introduction

Sea urchins and sand dollars, which comprise the echinoderm class Echinoidea, display a wide range of developmental modes. These include planktotrophic (indirect) and lecithotrophic (direct) free-swimming larvae, as well as brooded embryos (reviewed in Emlet *et al.*, 1987; Raff, 1987). Lecithotrophy has evolved independently in at least six orders of echinoids. Alterations in early echinoid development are tractable to experimental analysis, providing an excellent framework within which to analyze evolutionary changes in ontogeny (Raff *et al.*, 1989). To date, the best characterized direct developing echinoid is the Australian sea urchin *Heliocidaris erythrogramma* (Williams and Anderson, 1975). Despite the suffix, *Heliocidaris* is a euechinoid not a cidaroid. We have begun to analyze cellular and molecular alterations that accompany the evolution of direct development in this species (Parks *et al.*, 1988; Wray and Raff, 1989; Bisgrove and Raff, 1989). Predictions about ontogenetic alterations underlying direct development derived from this work can now be tested in echinoids that have independently evolved altered life history patterns.

Extant echinoids comprise two subclasses, which diverged during the Triassic: the Cidaroidea and the Euechinoidea (Smith, 1984a). The sizable literature on echinoid ontogeny deals almost exclusively with planktotrophic, or indirect, development in euechinoids. Comparatively few studies have examined development in cidaroids. Since cidaroids form the outgroup to euechinoids (Smith, 1984a), cidaroid ontogeny is important in assessing the polarity of ontogenetic transformations during echinoid evolution. Development in five planktotrophic cidaroids, three of them congeners, has been described: *Cidaris cidaris* (Prouho, 1887), *Prionocidaris baculosa* (Mortensen, 1938), *Eucidaris tribuloides* (Tennent, 1914, 1922; Schroeder, 1981; Wray and McClay, 1988), *E. metularia* (Mortensen, 1937), and *E. thouarsi* (Emlet, 1988). Although conforming to that of planktotrophic euechinoids in many regards, development in these cidaroids diverges in other respects (Emlet, 1988).

A variety of developmental modes has evolved in the

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Cidaroidea, yet only brief descriptions of direct development in cidaroids are available: brooding in *Goniocidaris umbraculum* (Barker, 1985) and lecithotrophic development in *Phyllacanthus parvispinus* (Raff, 1987) and *P. imperialis* (Olsen *et al.*, 1988). This report extends initial observations on *P. parvispinus*, and provides a detailed characterization of early development and metamorphosis in a cidaroid with lecithotrophic larvae. Since alterations in cell lineages have evolved in *H. erythrogramma* (Wray and Raff, 1989), particular attention is given to two differentiated cell types: skeletogenic mesenchyme cells and serotonergic neurons. Several aspects of *P. parvispinus* ontogeny are discussed with reference to the phylogeny of echinoids and the evolution of novel life history strategies.

Materials and Methods

Adult and embryo culture

Adult *Eucidaris tribuloides* were purchased from Carolina Biological. Adult *Phyllacanthus parvispinus* were collected at 2–10 m depth from rock crevasses near Sydney, New South Wales, Australia. Adults of both species were maintained in aquaria with circulating filtered seawater at 23°C for up to two weeks. *E. tribuloides* gametes were obtained, and embryos cultured, by standard methods (Hinegardner, 1967). Gametes of *P. parvispinus* were obtained by cutting open tests and teasing the gonads apart. Sperm of both species were collected "dry" and stored up to 24 h at 5°C; eggs were washed and immediately fertilized with dilute sperm suspensions. Embryos were cultured at low densities in unfiltered seawater at 23°C in 3 l glass beakers with gentle stirring. To encourage metamorphosis of *P. parvispinus*, some embryos were cultured singly in plastic tissue culture dishes (Costar). These dishes were "preconditioned" with seawater from aquaria holding adults for 2–3 days before addition of embryos. Formalin-fixed *Asthenosoma ijimai* embryos from Sagami Bay, Japan, were provided by Dr. Shonan Amemiya.

Light microscopy

Embryos were fixed for 1 h in 2% formalin in seawater, washed three times in Millipore-filtered seawater, and stored in 70% ethanol until prepared for examination. Some specimens were partially cleared by dehydration through a standard ethanol series into xylene, and mounted in Permount (Fisher Scientific). Other embryos were prepared for sectioning by dehydration and embedding in Paraplast (Monoject Scientific). Sections (6 μm) were stained with eosin and Harris hematoxylin according to standard methods (Lillie, 1965), or Alizarin red and methylene blue as in Parks *et al.* (1988). Live embryos were photographed using epi-illumination.

Total mesenchyme cell counts were obtained by staining 6-μm *P. parvispinus* sections with 0.3 mg/ml 4,6 Diamidino-2-phenylindole (DAPI) to visualize nuclei. Photographs were taken of a representative cross section and total mesenchyme nuclei counted. These numbers were used to extrapolate to whole embryo cell counts by comparing section volume to whole embryo volume. Msp130 positive cell numbers were obtained by staining serial sections with monoclonal antibody B2C2 (see below) and counting all labeled cells.

Immunohistochemistry

To reveal the distribution of the protein msp130, 6-μm paraffin sections were rehydrated and incubated with 10% normal goat serum in phosphate-buffered saline (PBS: 20 m*M* Na_2HPO_4, 140 m*M* NaCl, pH 7.6) for 30 min at room temperature, followed by a 1-h incubation in undiluted culture fluid containing monoclonal antibody B2C2 (Anstrom *et al.*, 1987). Sections were then washed in PBS, incubated in goat anti-mouse IgG-conjugated fluorescein isothiocyanate (Hyclone; diluted 1:100 in PBS), and washed. Serotonergic neurons were revealed by incubating whole embryos in Tris buffer (1% sodium metabisulfite, 0.05 *M* Tris, 1% sodium chloride) containing 0.3% Triton X-100 and 10% normal goat serum for 30 min at room temperature. This was followed by incubation in polyclonal rabbit anti-serotonin antibody (Incstar; 1:100 in Tris buffer) for 2 h at room temperature. Embryos were then washed in Tris buffer and incubated for 2 h in goat anti-rabbit IgG-conjugated fluorescein isothiocyanate (Hyclone; diluted 1:100 in Tris buffer). Stained embryos were washed and mounted in glycerol/PBS (7:3) containing 1.5% n-propylgallate for viewing.

Results

Gametes and fertilization

Neither males nor females of *Phyllacanthus parvispinus* could be induced to shed gametes by intracoelomic injection of 0.55 *M* KCl; eggs and sperm were obtained directly from the gonads. By gently dissecting apart the gonadal sheath, over 2 ml of eggs could easily be obtained from each female. The reproductive season of *P. parvispinus* found in the Sydney area is apparently restricted to February and March. Even during that time, some morphologically mature batches of eggs did not fertilize.

P. parvispinus eggs are approximately 700 μm in diameter (Mortensen, 1921), much larger than those of planktotrophic cidaroids (90–170 μm; Emlet *et al.*,

Table I

Developmental timecourses among echinoids

	Species: *P. parvispinus* Subclass: Cidaroidea Larvae: lecithotrophic	*E. tribuloides* Cidaroidea planktotrophic	*H. erythrogramma* Euechinoidea lecithotrophic	*H. tuberculata* Euechinoidea planktotrophic
Stage:				
2-cell	1.5	1.5	1.5	1.5
8-cell	2.5	2.5	2.5	2.5
Wr. blastula	8–12	—	6–9	—
Blastula	13–15	7–13	10–11	6–13
Early gastr.	18	18	17	16
Mes. ingress.	??	23	12	14
Late gastr.	30	40	20	20
Coeloms	33	50	22	22
Prism	—	55	—	24
Pluteus	—	90	—	39
Rudiment	37	(25 days)	30	??
Metamorphosis	5 days	(30 days)	3.5 days	??

Developmental timecourses for representative lecithotrophic and planktotrophic developers from the Cidaroidea and Euechinoidea are listed for purposes of comparison. Note that stages being compared between lecithotrophic and planktotrophic larvae are similar but not literally equivalent, because some developmental events have undergone temporal shifts (heterochronies). Approximate times required to reach the listed stages of development at 23°C are in hours unless otherwise noted (bracketed time points in second column are based on *E. thouarsi* reared at 28°C); approximate durations are provided for blastulae. Dashes indicate absence of a particular stage; question marks indicate stages whose timing is not known. In the two euechinoids, mesenchyme ingression preceeds gastrulation, while the reverse is true of *E. tribuloides;* when mesenchyme cells first ingress in *P. parvispinus* remains to be determined. Sources: *Phyllacanthus parvispinus* (this study); *Eucidaris tribuloides/E. thouarsi* (Schroeder, 1983; Emlet, 1988; Wray and McClay, 1988); *Heliocidaris erythrogramma* (Williams and Anderson, 1975; Parks *et al.,* 1988); *Heliocidaris tuberculata* (Parks *et al.,* 1988). Abbreviations: wr. blastula = wrinkled blastula; gastr. = gastrula; mes. ingress. = initial mesenchyme cell ingression; coeloms = initiation of coeloms; pluteus = 2-armed pluteus.

1987). The buoyant eggs are gray and opaque, and possess no visible asymmetries in pigment distribution. As with other direct developing echinoids, sperm heads are longer and narrower than those of related planktotrophic species (Raff *et al.,* in prep).

Early development

Table I presents a timecourse of developmental events in *P. parvispinus.* Early development of *P. parvispinus* proceeds at approximately the same rate as that of a planktotrophic cidaroid species, *Eucidaris tribuloides.* Beginning with the formation of adult structures, however, *P. parvispinus* develops much more rapidly, resulting in a juvenile within 5 days instead of a month. This parallel timing of early development followed by acceleration of metamorphosis is also characteristic of euechinoid lecithotrophic development (Table I).

Zygotes cleave approximately 90 min after fertilization; thereafter, cleavage divisions occur at about 35-min intervals. Early cleavages are essentially equal, with slight variations in blastomere size apparently arising at random. During fourth cleavage (2.8 h), transient 12-cell embryos are occasionally observed. The 16-cell embryo (3 h) is composed of two tiers of eight blastomeres of approximately equal size (Fig. 1a). During fifth cleavage (3.5 h), some 20–30 cells are present; blastomeres at one end of the embryo are distinctly smaller than those at the other end (Figs. 1b, 2a). Together, these observations suggest that cleavages become asynchronous as early as the fourth division, with blastomeres at one pole of the embryo cleaving at a slightly faster rate. This stands in contrast to another direct developing echinoid, *H. erythrogramma,* which has synchronous, equal cleavage through at least the seventh division (Wray and Raff, 1989).

The blastular epithelium of *P. parvispinus* undergoes a pronounced wrinkling during the next several hours (Fig. 1c). The formation of wrinkled blastulae is a common feature in direct developing echinoderms with large eggs (Mladenov, 1979; Strathmann, 1987). Wrinkled blastulae also occur in the lecithotrophic euechinoids *Heliocidaris erythrogramma* (Williams and Anderson, 1975), *Holopneustes inflatus* (V. B. Morris, unpub. obs.), and *Asthenosoma ijimai* (Amemiya and Tsuchiya, 1979). Both depth and number of wrinkles in *P. parvispinus* reach a maximum at about 10 h (Figs. 2b, 3a). Wrinkles differ from those of other direct developing echinoderms in their depth, which often exceeds the radius of the blastula, and in the occurrence of branches

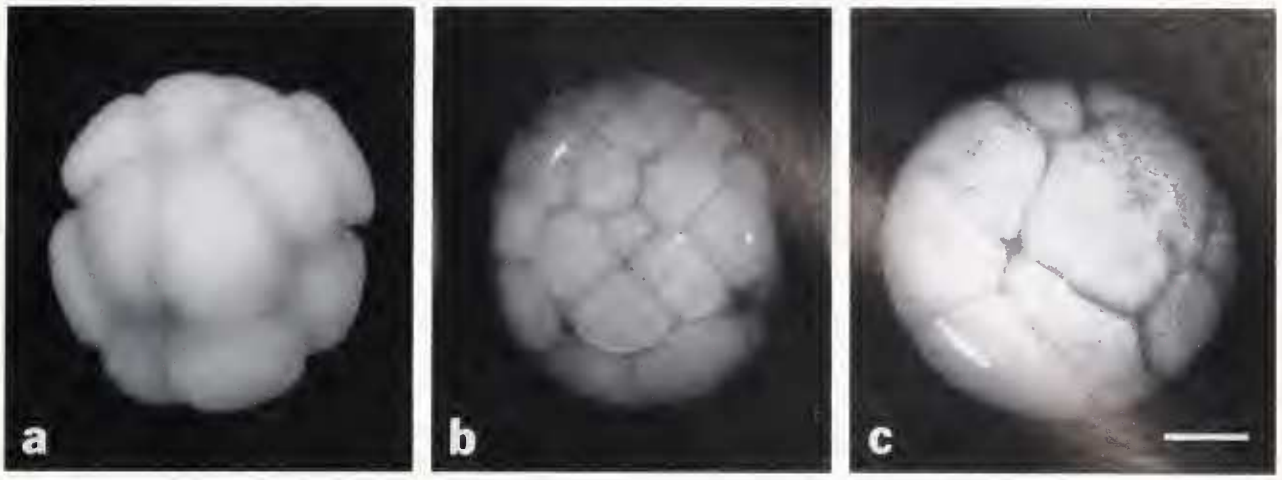

Figure 1. Early development of *Phyllacanthus parvispinus*, whole mounts of fixed and live embryos. a. 16 cell fixed embryo (3 h). The first four cleavages are equal. b. Approximately 30-cell live embryo (3.5 h). Note blastomeres are of different sizes. c. Live wrinkled blastula (11.5 h). At this stage, the wrinkles are decreasing in number. The embryo is still inside the fertilization envelope.

(Fig. 3a). At this stage, the epithelium is composed of cuboidal cells. Over the next several hours, wrinkles gradually egress in concert with a distinct increase in epithelial thickness. By 13 h, the blastula is lobate and irregular in appearance, and most embryos have only one or two very deep wrinkles remaining (Fig. 2c). The epithelium at 13 h is columnar, and approximately twice as thick as at 10 h (compare Figs. 3a and 3b). Throughout the appearance and disappearance of wrinkles, the diameter of the blastula does not change appreciably; it remains within the fertilization envelope. A similar situation exists in *H. erythrogramma* (Williams and Anderson, 1975).

Wrinkles have completely disappeared from the surface of the embryo by the beginning of gastrulation. At 21 h, the archenteron extends approximately one third of the distance across the blastocoel. The archenteron is unusually wide for an echinoid. Many mesenchyme cells are present by this time. The tip of the archenteron begins to widen into the presumptive coelom by 26 h (Figs. 2d, 3c). Archenteron elongation then ceases, and the coelom continues to elaborate over the next several hours. The 33-h larva contains a bilobed coelom, one side of which can be distinguished as hydrocoel and is already branching into five buds (Fig. 3d), marking the beginning of morphogenesis of the echinus rudiment.

An unusual feature of larval development in *P. parvispinus* is the presence of cylindrical pits, up to 100 μm deep, opening onto the ectodermal surface (Figs. 2f and 3c, arrows). These pits, which number about four to six per embryo, seem to be distributed randomly. It is not clear how pits arise, but it seems unlikely that they are remnants of the one or two wrinkles present in lobate blastulae because of their greater number and distribution over the whole of the embryo. Pits are present through metamorphosis (Fig. 2f, arrow).

Larval development in *P. parvispinus* occurs near the air and water interface. Embryos hatch from the fertilization envelope at about 18 h, just prior to the beginning of gastrulation. Hatched larvae float with the animal end up; the adult oral-aboral axis is oriented perpendicular to the embryonic animal-vegetal axis. Larvae spin slowly about the animal-vegetal axis. *P. parvispinus* larvae are uniformly ciliated and lack a ciliary band. Unlike most other echinoid larvae, these embryos do not swim in a directed manner in culture.

Development of echinus rudiment and metamorphosis

By about 37 h, a cluster of five podial buds is evident on the lateral larval ectoderm. These nascent primary podia (tube feet) surround the location of the future adult mouth and define the adult rudiment; they are the first external manifestation of pentamerous adult symmetry. During the next several hours, podia grow rapidly, and by 47 h are beginning to achieve their final form (Figs. 2e, 3e). As reported previously (Raff, 1987), no ectodermal invagination forms a vestibule around the adult rudiment of *P. parvispinus* (Fig. 3e, f). By 48 h, larval ectoderm contains numerous, brightly pigmented cells.

The floating larva becomes denser as larval development proceeds. On the fourth day of development, it sinks and begins to move about on the substratum using its primary podia. The first spines are composed of three parallel rods interconnected by short crossbridges. These are juvenile spines based on their development of charac-

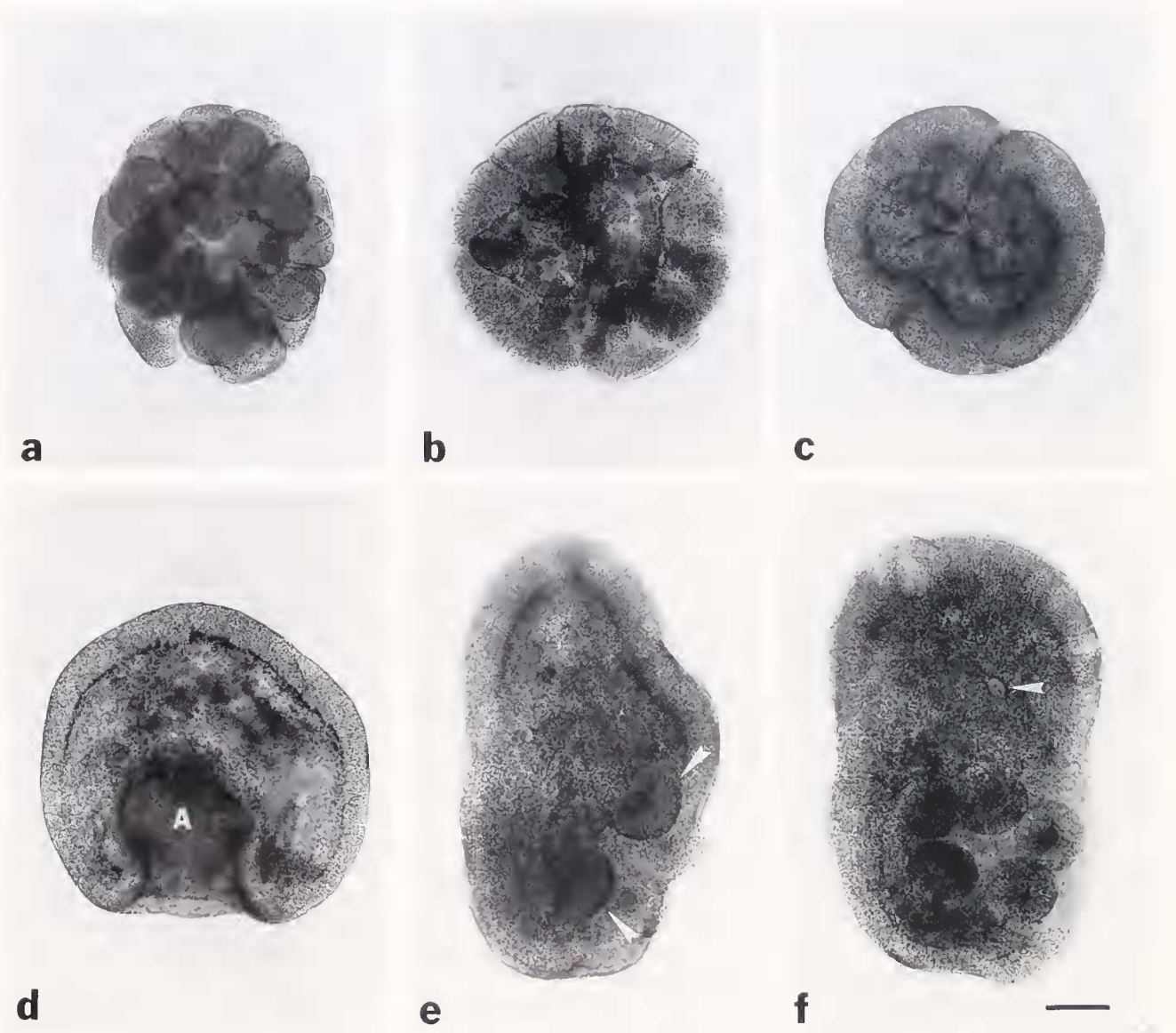

Figure 2. Development of *Phyllacanthus parvispinus*, xylene cleared whole mounts. a. Approximately 30-cell embryo (3.5 h). Note size differences in blastomeres. b. Wrinkled blastula (10 h). The surface is maximally wrinkled at this stage. c. Lobate blastula (13.5 h). Only a few wrinkles remain at this stage. d. Gastrula (26.5 h). The blastopore is quite wide. e. Early larva (45 h). Podial buds are present (arrows). f. Late larva (69 h). The five primary podia surrounding the future adult oral surface are clearly visible, as is a pit opening (arrow). Embryos in panels d–f are oriented animal end up. Formalin-fixed embryos were cleared in xylene before micrography. A, archenteron. Scale bar = 100 μm.

teristic flared ends, and on the fact that they are the first spines to appear. However, these spines differ from the juvenile spines of planktotrophic cidaroids in their cluster arrangement on the test and in the fine structure of the lateral processes (Emlet, 1988). Pedicellaria are not yet present, and a large lobe, corresponding to the animal end of the larva, protrudes from one side. During the next few days, metamorphosis is gradually completed: the larval animal lobe is resorbed, circumoral spines develop the characteristic lateral processes and flared ends of echinoid juvenile spines, and additional spines appear. By 141 h, the surface is covered by juvenile spines, and five apical test plates are evident (Fig. 4). At this point, test diameter is approximately 500 μm.

Although juveniles at six days of development contain nascent adult test plates, there is no evidence of calcification in the adult oral field. It is not known when juveniles begin to feed, but development of the lantern and

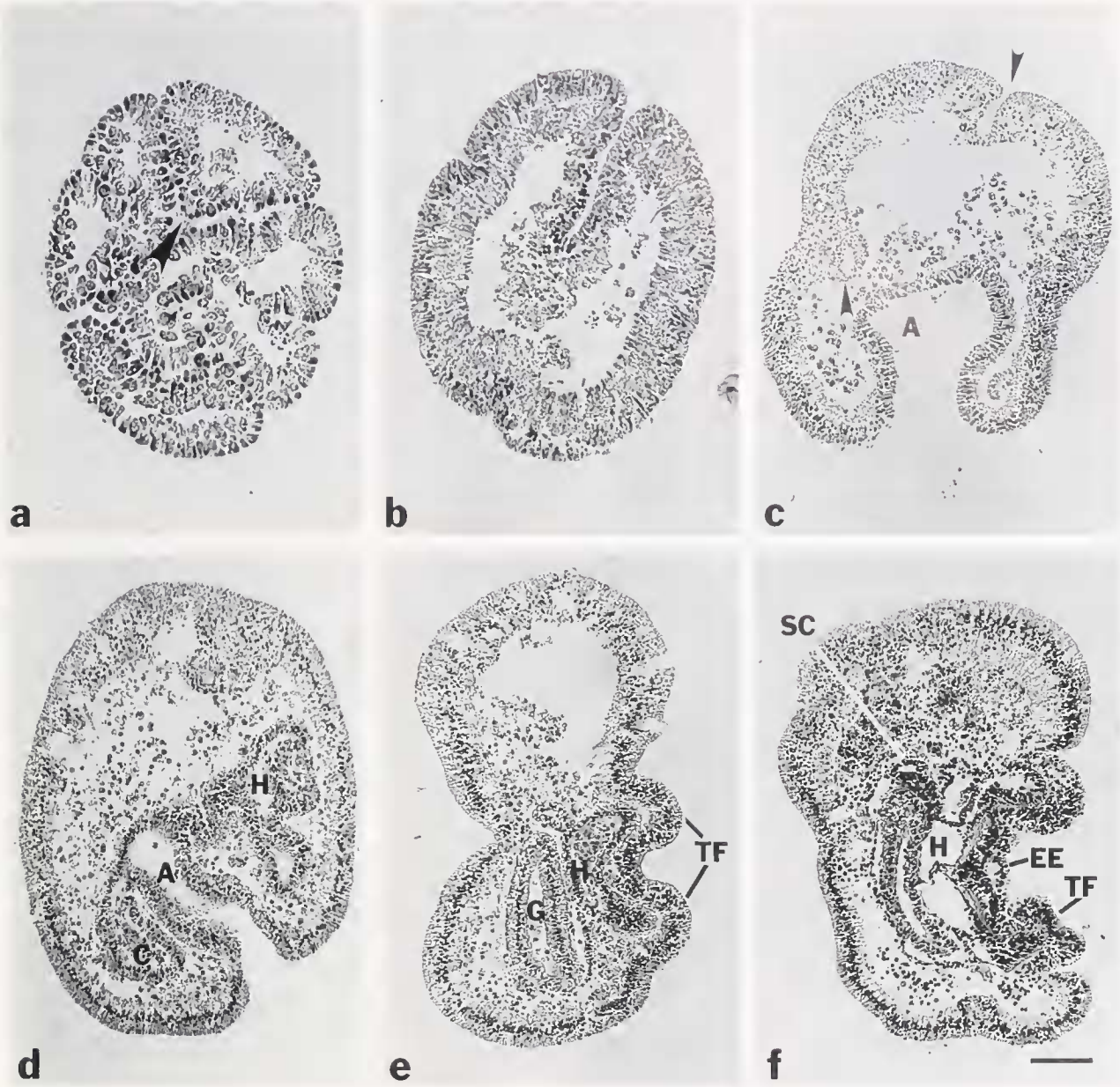

Figure 3. Development of *Phyllacanthus parvispinus*, sections. a. Wrinkled blastula (10 h). The highly convoluted surface is composed of loosely organized epithelium. A branchpoint is labeled (arrow). b. Lobate blastula (13.5 h). A single, deep wrinkle is visible; ectoderm is now much thicker. c. Gastrula (26.5 h). Numerous mesenchyme cells have ingressed from the tip of the archenteron (A). Note pits (arrows). d. Late gastrula (33 h). Mesenchyme cell ingression is complete and the coelom (C) and hydrocoel (H), a coelomic derivative, are forming. e. Early larva (45 h). Section passes through the adult rudiment and two primary podia (TF). Branches of the hydrocoel invest the podial buds. Note the lack of a vestibule enclosing the rudiment. f. Late larva (95 h). Podia have terminal discs and will support locomotion. Micrographs are of 6-μm paraffin sections prepared from formalin-fixed embryos and stained with eosin and Harris hematoxylin. Embryos in panels c–f are oriented with the animal pole at the top of the photo. EE, epineural epithelium; G, gut; SC, stone canal. Scale bar = 100 μm.

teeth evidently occurs some time after completion of most other metamorphic events. Pedicellaria and definitive adult spines are not present in six day juveniles (Fig. 4), but both are evident a week later (not shown). These later spines lack the characteristic flared ends of earlier juvenile spines.

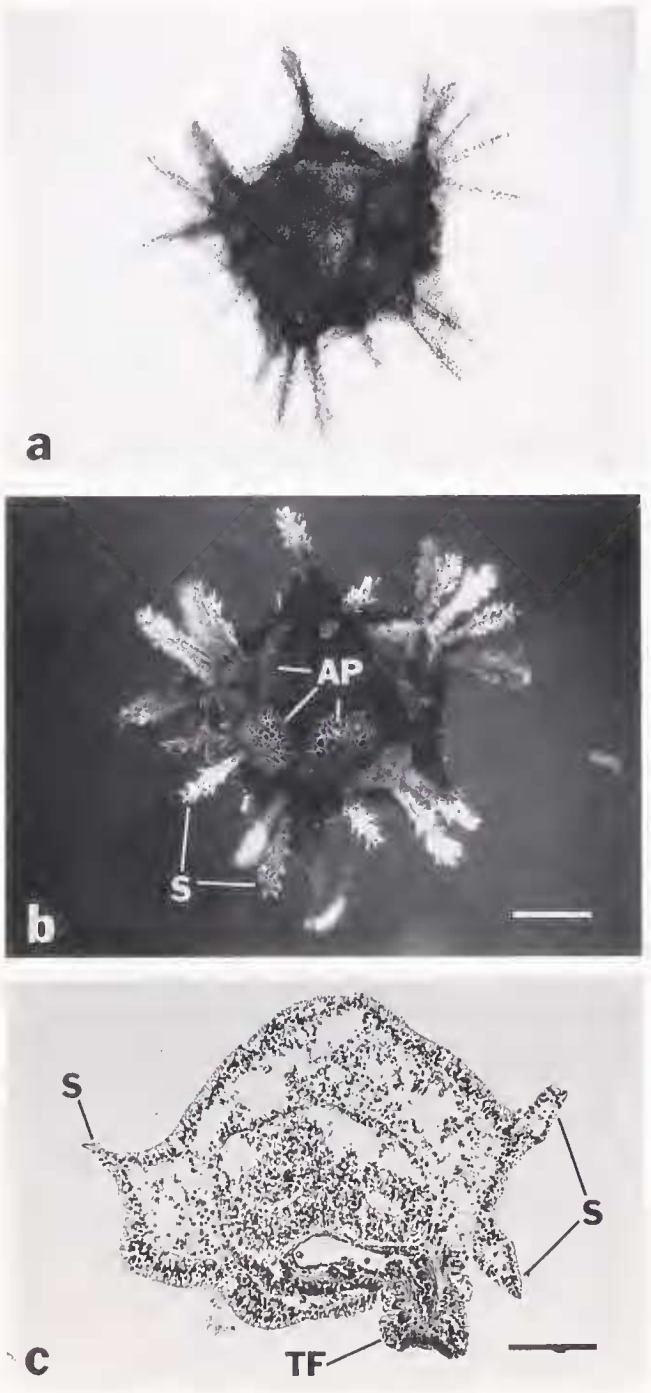

Figure 4. Juvenile *Phyllacanthus parvispinus.* Three views of 141-h juveniles. a. Xylene-cleared whole mount, aboral view. Note "webbing" between spines. b. Polarized light view of the same specimen. Secretion of the five apical plates (AP) has begun; three are visible in this phase of polarized light. Spines (S) are all of the juvenile type. c. Six-μm eosin and Harris hematoxylin cross section. A single podium (TF) is visible, as are three spines. The section is oriented oral surface down. Scale bars: a, b = 200 μm; c = 150 μm.

The larval ectoderm of planktotrophic euechinoids is largely lost during metamorphosis, the adult ectoderm deriving primarily from vestibular ectoderm (Cameron and Hinegardner, 1978). In contrast, the larval ectoderm of the cidaroid *Eucidaris thouarsi* is retained through metamorphosis (Emlet, 1988). Indirect evidence suggests that a similar situation exists in *P. parvispinus.* The ectoderm is topologically in the same position in late larvae and juveniles, because no inversion of a vestibule takes place. Larval ectoderm retracts onto the aboral surface of the juvenile as metamorphosis proceeds (Figs. 4a, c).

Differentiation of mesenchyme cells

The differentiation of skeletogenic mesenchyme cells and the pattern of skeletogenesis is highly modified in lecithotrophic echinoids (Raff, 1987; Parks *et al.,* 1988). The protein msp130 provides a specific probe for these cells. This protein is produced only by primary mesenchyme cells in euechinoid embryos (Anstrom *et al.,* 1987; Wray and McClay, 1989) and by skeletogenic cells in adults (Parks *et al.,* 1988). In planktotrophic larvae of the cidaroid *Eucidaris tribuloides,* there are 16 spicule-forming cells, homologous to euechinoid primary mesenchyme cells, that express msp130 (Wray and McClay, 1988). In *E. tribuloides,* msp130 expression begins after spicule-forming cell ingression is complete, and just before secretion of the larval skeleton.

To examine expression of msp130 during *P. parvispinus* development, embryos were stained using indirect immunofluorescence with the monoclonal antibody B2C2, which binds specifically to msp130 (Anstrom *et al.,* 1987). In early gastrulae containing hundreds of mesenchyme cells (22 h), no staining is apparent. By the time coeloms are elaborating (33 h), cell counts reveal that there are 6,000–10,000 mesenchyme cells present, of which approximately 5% exhibit B2C2 staining (Fig. 5a, b). In premetamorphic larvae, B2C2-positive cells are closely associated with calcareous spicules being elaborated into juvenile spines and test stereom (Fig. 5c, d). By 95 h, B2C2-positive cells are also present in podial terminal discs and the oral region, where additional spicule synthesis will take place. Only those mesenchyme cells adjacent to, and probably participating in, synthesis of the calcareous spicules are B2C2 positive (compare Figs. 5d and 5f).

Larval serotonergic nervous system

The evolution of direct development in several echinoids results in the reduction of structures at the apical end of the pluteus, including the larval arms and the circumoral ciliary band. *P. parvispinus* larvae lack both arms and a ciliated band. The pluteus' serotonergic ner-

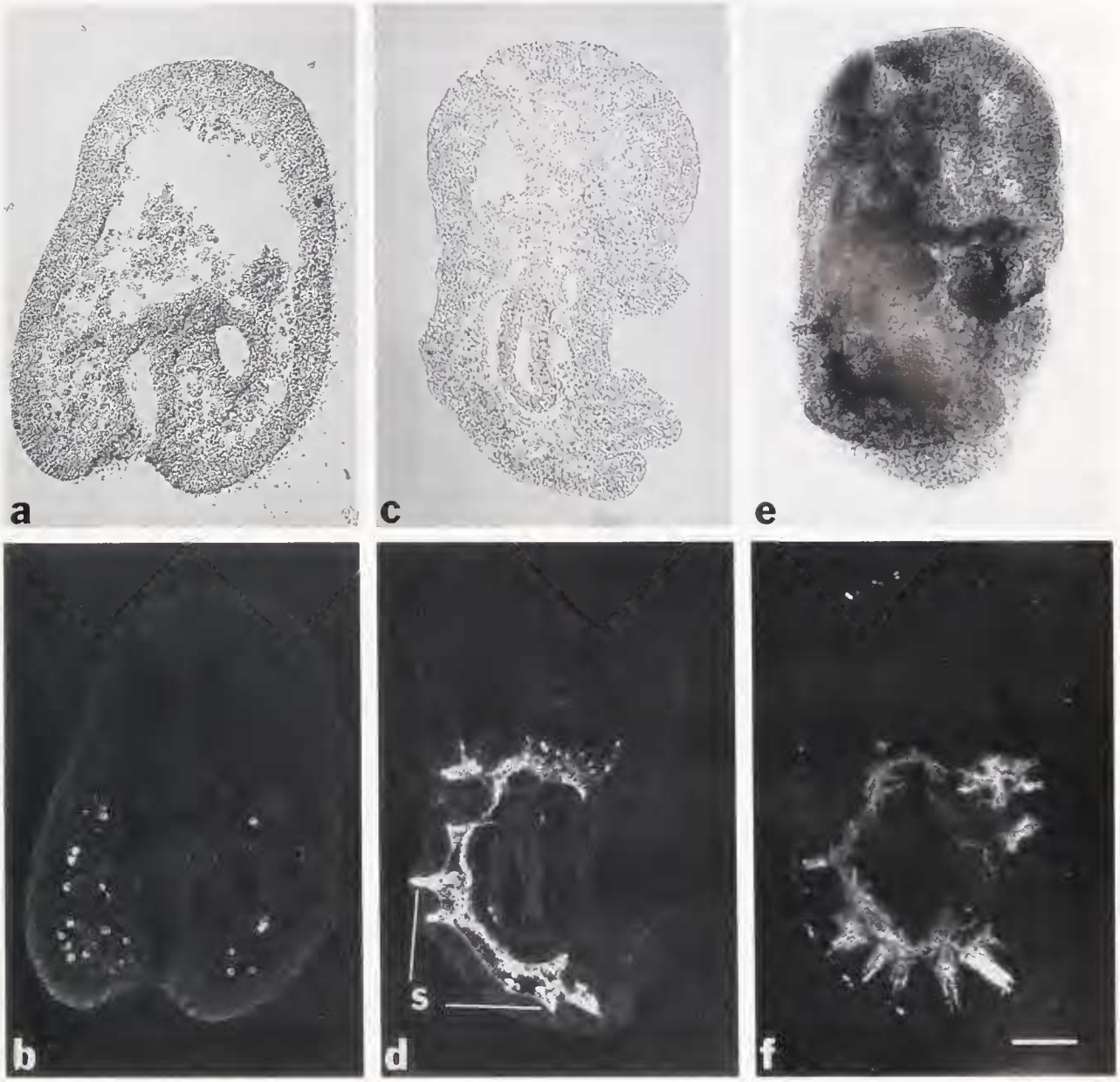

Figure 5. msp130 expression. a–d. Indirect immunofluorescent staining of sectioned embryos with monoclonal antibody B2C2. a, b. Paired brightfield and fluorescent micrographs of late gastrula (33 h). A scattering of positive cells are present in the vegetal end of the embryo. c, d. Paired brightfield and fluorescent micrographs of late larva (95 h). Many more positive cells are present. Staining is strong near nascent juvenile spines (S) on adult aboral side (left in these panels). Staining is also present in podia and adult oral region (not shown). e, f. Paired brightfield and polarized light micrographs of a late larva (95 h) xylene-cleared whole mount. Embryo is oriented with the future adult oral field facing the viewer. This larva shows the extent and position of adult test and spine secretion for comparison with the specimen in panels c and d. Scale bar = 100 μm.

vous system, which has an unknown function, also lies at the apical end of the pluteus larva. Serotonergic neurons provide an identifiable cell lineage that has been used to document echinoid nervous system development. These studies have also allowed comparisons of temporal and morphological variation in nervous system differentiation among planktotrophic and lecithotrophic species. We asked if these neurons are reduced or modified in *P. parvispinus*.

Development of larval serotonergic neurons in *P. par-*

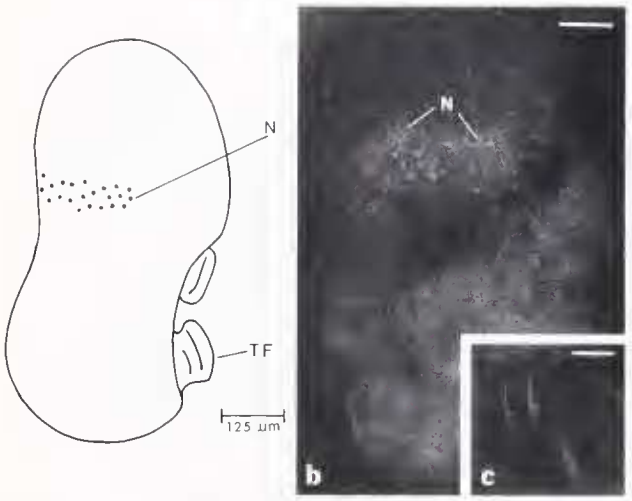

Figure 6. Distribution of serotonergic neurons in *P. parvispinus* larvae (114 h). a,b. The cluster of serotonergic neurons (N) is located in the larval epidermis opposite the echinus rudiment. Larva contains 30–50 loosely clustered neurons located medially in the larval epidermis opposite to the rudiment. View of larva in b is of side directly opposite rudiment. c. High magnification view of the neurons from b showing cell bodies and apical processes. TF, tube foot. Scale bars: b = 100 μm; c = 25 μm.

vispinus was studied using a polyclonal antibody that binds specifically to serotonergic cells. Anti-serotonin immunoreactive cells could be resolved in 92 h and older larvae. In these larvae, 30–50 serotonergic cells are present, loosely clustered in the epidermis of the larva, opposite to the oral field of the adult rudiment (Fig. 6). The serotonergic neurons are 9 μm (basal diameter) flask-shaped cells with a basal nucleus and a long apical process (about 20 μm) that extends to the surface of the epidermis (Fig. 6c). One or two axons project basally from the neurons; the organization of the axons is difficult to resolve through the thick larval epidermis. Although we were unable to resolve serotonergic neurons in larvae younger than 92 h, these cells presumably arise earlier than we have documented. This organization is quite different from the larval serotonergic nervous systems of both planktotrophic and lecithotrophic euechinoids (Bisgrove and Burke, 1986, 1987; Nakajima, 1987; Bisgrove and Raff, 1989), which have paired, interconnected clusters of neurons at the animal end of the larva.

Because the presence and location of a serotonergic nervous system has not been previously demonstrated in planktotrophic cidaroids, larvae of a planktotrophic cidaroid, *Eucidaris tribuloides*, were stained to reveal the serotonergic nervous system for comparison. In early plutei (8 day), six immunopositive cells are present; the number and location of neurons (Fig. 7a) was almost invariant among larvae of the same age despite some variation in morphological development. These 8–9 μm (basal diameter) cells lie within the epidermis along the base of the circumoral ciliary band. Bilaterally symmetrical pairs of neurons occur at the ventrolateral margins of the preoral hood, and a single cell lies at the base of each postoral arm (Fig. 7b, c). An axon up to 40 μm long extends from each neuron at the base of the postoral arms along the ciliary band toward the ipsilateral neurons in the preoral hood (Fig. 7c, d). Short (5–7 μm), axons extend, in no preferred direction, from the neurons in the preoral hood.

The vestibule in direct developing echinoids

Planktotrophic euechinoids possess a vestibule, or amniotic invagination, formed from the ectoderm overlying the hydrocoel (Hyman, 1955). This feature is absent from *Eucidaris thouarsi*, the only indirect developing cidaroid whose metamorphosis has been carefully described (Emlet, 1988). No vestibule is formed by *P. parvispinus* (Fig. 3), which is consistent with its position as a cidaroid.

A vestibule is present in euechinoid direct developers *H. erythrogramma* (Parks *et al.*, 1988), *Peronella japonica* (Okazaki, 1975), *Holopneustes inflatus* (Henry and Raff, unpub. obs.), and *Abatus cordatus* (Schatt, 1985). Thus the vestibule is apparently not lost as a consequence of direct development. To cast light on the phylogenetic origin of this feature in echinoid evolution, we re-examined metamorphosis in *Asthenosoma ijimai*, an echinothurioid. The published description of *A. ijimai* development (Amemiya and Tsuchiya, 1979), which carefully documents external features, shows no vestibule. We have serially sectioned *A. ijimai* larvae (Fig. 8) for direct comparison with *P. parvispinus*, and find no trace of an ectodermal vestibular invagination.

Discussion

Development in *Phyllacanthus parvispinus* diverges in several respects from "typical" echinoid ontogeny. Differences have arisen throughout development in molecular and cellular processes, as well as in morphological and behavioral features. Some differences are likely common to all cidaroids, and date to the evolutionary separation of cidaroids and euechinoids during the Triassic. Other differences may represent evolutionary novelties associated with the evolution of a lecithotrophic developmental mode. In this discussion, we attempt to distinguish between developmental patterns in *P. parvispinus* that result from phylogenetic history and those that result from abbreviated development.

Cidaroid early development

The few planktotrophic cidaroids whose development has been studied share a set of developmental features

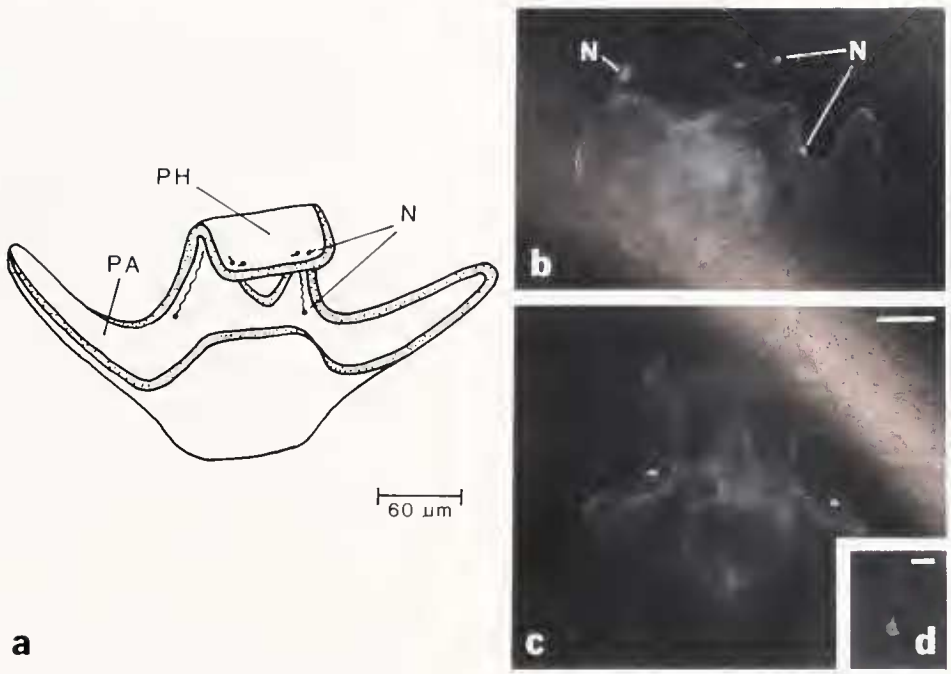

Figure 7. Distribution of serotonergic neurons in *E. tribuloides* early pluteus larvae (8 day). a. Larvae at this stage contain six bilaterally distributed serotonergic neurons (N). A single neuron lies near the base of the postoral arms, and extends an axon to a pair of neurons at the top of the preoral hood; no connections between contralateral axons are present. In all panels, larvae are oriented animal end up. b. Ventral view; a pair of neurons in the preoral hood and a single neuron at the base of the postoral arm are visible on the left side of the larva. c. Dorsal view showing the bilaterally symmetric arrangement of neurons at the base of the postoral arms. Each of these neurons (shown at higher magnification in d), extends an axon toward the preoral hood. PA, postoral arms; PH, preoral hood. Scale bars: b, c = 40 μm; d = 10 μm.

that distinguish them from euechinoids. These features, which have been documented for one or more species, include: a relatively thin hyaline layer; absence of maternal α-subtype histone mRNA in the egg; a variable number of micromeres at the 16-cell stage; lack of an apical tuft following hatching; absence of mesenchyme cell ingression prior to gastrulation; relatively slow development; several morphological features of the pluteus larva; and the lack of a vestibule during echinus rudiment formation (Prouho, 1887; Tennent, 1914, 1922; Mortensen, 1937, 1938; Schroeder, 1981; Raff *et al.*, 1984; Emlet, 1988; Wray and McClay, 1988). Planktotrophic euechinoids differ from cidaroids in each of these features (reviewed in Okazaki, 1975, and Emlet, 1988).

Direct developing euechinoids display several modifications from planktotrophic euechinoid development. These include: larger, yolky eggs; larger, more elongate sperm heads; alterations in the geometry of the fourth cleavage; transient "wrinkling" of the blastula; reduced or absent larval skeleton; and a general acceleration of adult rudiment formation (Okazaki, 1975; Williams and Anderson, 1975; Amemiya and Tsuchiya, 1979; Raff, 1987; Emlet *et al.*, 1987; Parks *et al.*, 1988; Bisgrove and Raff, 1989; Wray and Raff, 1989; Raff *et al.*, in prep.). Because planktotrophic development via a pluteus larva is likely a primitive feature in post-Paleozoic echinoids (Strathmann, 1975, 1978; Emlet *et al.*, 1987; Raff, 1987), ontogenetic alterations shared by direct developers in separate orders are derived features, and constitute parallelisms (Raff *et al.*, 1989; Wray and Raff, 1989).

P. parvispinus displays developmental features typical of cidaroids: development is slower than in euechinoids with a comparable developmental mode (Table I), and there is no vestibule present in the larvae. Because the described lecithotrophic euechinoids do not share these characters, it is probable that they are the result of phylogenetic history and not adaptations peculiar to lecithotrophic development. We expect that additional features characteristic of cidaroid development, such as the absence of mesenchyme cell ingression prior to gastrulation, will also be found in *P. parvispinus* upon further examination.

It is also significant that in many regards *P. parvispinus* conforms to the general characteristics of echinoid lecithotrophic development: elongate sperm heads, large eggs, lack of micromeres at the 16-cell stage, a transient wrinkled blastula, lack of a larval skeleton, and heterochronies in morphogenesis. Because these are aspects of

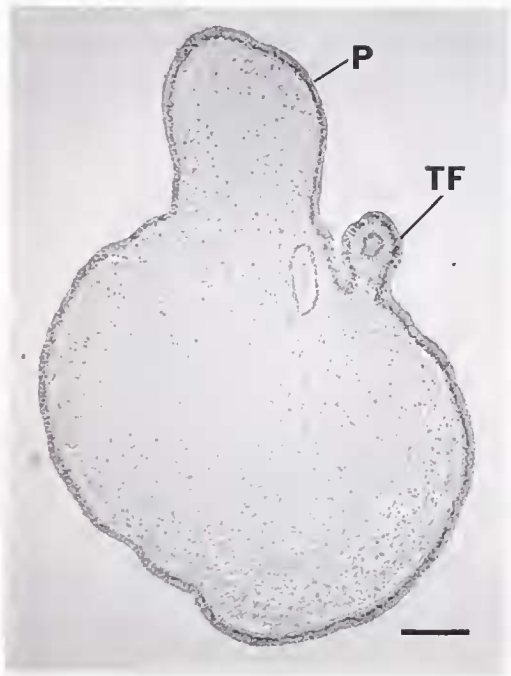

Figure 8. *Asthenosoma ijimai* lacks a vestibule. 6 μm Alizarin red and methylene blue stained section of a 101-h *A. ijimai* larvae showing one psuedo arm (P) and a developing tube foot (TF). Embryos of several stages (35, 45, 56, 75, and 101 h) were serial sectioned and no evidence of a vestibule was found at any stage across the developing adult rudiment. Scale bar = 150 μm.

early development in lecithotrophic echinoids of three, independently evolved euechinoid lineages [Echinometridae, Echinothuriidae, Temnopleuridae (Raff, 1987)], they are likely to represent functional adaptations to, or are consequences of, a change in developmental mode. This hypothesis is strengthened by the fact that these features are not characteristic of planktotrophic development in either cidaroids or euechinoids.

Skeletogenic mesenchyme cells and expression of msp130

We can distinguish three distinct classes of mesenchyme cells in the *P. parvispinus* larva: skeletogenic and nonskeletogenic mesenchyme (msp130 positive and negative) cells in the blastocoel, and pigment cells inserted in the ectoderm. Indirect developing cidaroid and euechinoid embryos contain these same three classes of mesenchyme cells (Gibson and Burke, 1985; Wray and McClay, 1988).

One of the most striking features of lecithotrophic development in *Heliocidaris erythrogramma*, a euechinoid, is the elimination of the larval pattern of skeleton formation and its replacement by an accelerated adult skeleton assembly. The expression pattern of msp130, a protein produced in larval and adult spicule-forming cells of echinoids, is also altered in direct developing echinoids (Parks *et al.*, 1988). In planktotrophic euechinoid larvae, msp130 is first expressed at, or some time before, synthesis of the larval skeleton (Wray and McClay, 1989). In *H. erythrogramma*, msp130 expression is delayed relative to that in planktotrophic larvae: expression commences hours after mesenchyme cell ingression, and is concurrent with the initiation of the echinus rudiment (Parks *et al.*, 1988). Expression of msp130 in *P. parvispinus* follows a similar timecourse. It seems likely that expression of msp130 and spicule synthesis have undergone parallel changes in these two independently derived lecithotrophic larvae: the larval program of spicule synthesis has been excised, and the adult program is initiated earlier relative to coelom formation and accelerated.

Echinoid larval nervous systems

Serotonergic nervous systems in planktotrophic euechinoids from two families have been characterized (Stronglyocentrotidae and Echinometridae; Bisgrove and Burke, 1986, 1987; Bisgrove and Raff, 1989). Although it has not been characterized in detail, the nervous system of a planktotrophic cidaroid, *Eucidaris tribuloides*, described here differs substantially from that of planktotrophic euechinoids. At a comparable stage of development (2-armed pluteus), *E. tribuloides* has fewer neurons than euechinoid plutei, the number of neurons is almost invariant, and neurons in the preoral hood lack long axonal processes extending between contralateral clusters. In addition, neurons are present at the base of the postoral arms, whereas in euechinoids, serotonergic neurons are confined to the preoral hood. Additional data will be required to characterize more fully cidaroid larval serotonergic nervous systems, but differences have clearly arisen since the euechinoids and cidaroids diverged.

The position and distribution of the larval serotonergic nervous system in *P. parvispinus* differs from that in *E. tribuloides* as well as planktotrophic euechinoids in that neurons are located medially in a single large cluster rather than as bilaterally symmetric clusters at the animal end of the larva. The *P. parvispinus* larval nervous system also differs from that of *Heliocidaris erythrogramma*, a euechinoid with lecithotrophic development (Bisgrove and Raff, 1989). In *H. erythrogramma*, serotonergic neurons are organized in two large, interconnected clusters at the animal end of the larva. Therefore, the altered organization and position of the larval nervous system in *P. parvispinus* is not solely a consequence of lecithotrophic larval development as it has been modified in ways distinct from those observed in *H. erythro-*

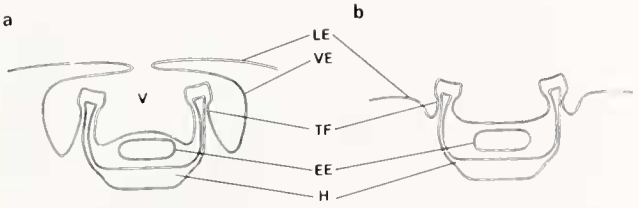

Figure 9. Comparison of adult rudiment in echinoids. Diagrammatic cross sections through the rudiment of a non-echinothurioid euechinoid (a) and a cidaroid or echinothurioid euechinoid (b). EE, epineural epithelium; H, hydrocoel; LE, larval ectoderm; TF, tube foot; V, vestibule; VE, vestibular ectoderm. Summarized from: Raff (1987), Emlet (1988), and this study (Cidaroidea); Amemiya and Tsuchiya (1979) and this study (Echinothurioida); and Okazaki (1975), Cameron and Hinegardner (1978), and Parks *et al.* (1988) (other Euechinoidea).

gramma. The orientation of the cluster of serotonergic neurons with respect to the oral-aboral axis of the echinus rudiment in *P. parvispinus* is also unusual. In *E. tribuloides* and the planktotrophic and lecithotrophic euechinoids that have been examined, the axis along the clusters of neurons lies oblique to the oral-aboral axis of the echinus rudiment, not perpendicular to it, as appears to be the case in *P. parvispinus.* The significance of the modifications in position, distribution, and orientation of the nervous system in *P. parvispinus* remains unknown.

Cidaroid metamorphosis

The adult rudiment in euechinoid sea urchins arises from the left coelomic pouch, which produces the hydrocoel (Bury, 1895; Fukushi, 1959, 1960; Cameron and Hinegardner, 1978; Okazaki, 1975). The vestibule forms as an invagination of larval ectoderm over the hydrocoel. Vestibular ectoderm encloses the adult rudiment, and its floor eventually becomes adult oral epithelium (Fig. 9a). It should be noted that analogous structures termed "vestibules" are present in some members of other echinoderm classes (Hyman, 1955), but cannot be considered homologous to the vestibules of euechinoids (Emlet, 1988).

The only cidaroid whose metamorphosis has been described in detail is *Eucidaris thouarsi,* a species with planktotrophic larvae (Emlet, 1988). As in euechinoids, the adult rudiment of *E. thouarsi* arises from the left coelomic pouch; however there is no vestibule (Fig. 9b). Metamorphosis in *E. thouarsi* is also distinguished from that of euechinoids by the presence of numerous juvenile spines on the echinus rudiment and retention of the larval ectoderm.

There is no vestibule present in *P. parvispinus,* nor in its congener, *P. imperialis,* which also undergoes lecithotrophic development (Olsen *et al.,* 1988). The absence of a larval mouth in *P. parvispinus* makes it unclear whether the adult rudiment arises exclusively from the left coelomic pouch. Metamorphosis in *P. parvispinus* is gradual, and like *E. thouarsi,* many juvenile spines are present, and much of the larval ectoderm appears to be retained. These similarities in mode of metamorphosis may prove characteristic of cidaroids in general.

The Echinothurioida, usually considered the most primitive euechinoid order (Jensen, 1981; Smith, 1984a), is the only euechinoid order in which all described species lack a vestibule. In the echinothurioid *Asthenosoma ijimai,* the adult rudiment develops on the larval surface, and is never surrounded by a vestibule (Amemiya and Tsuchiya, 1979; this paper). Since all echinothurioids appear to be direct developers (Emlet *et al.,* 1987), it has not been clear whether this is an adaptation for lecithotrophic development or a primitive feature of echinoid ontogeny. However, the presence of a vestibule in four independently evolved direct developing euechinoids (*Peronella japonica,* Okazaki and Dan, 1954; *H. erythrogramma,* Parks *et al.,* 1988; *Abatus cordatus,* Schatt, 1985; *Holopneustes inflatus,* Henry and Raff, unpub. obs.), and its absence in *E. thouarsi* demonstrates that loss of the vestibule is not a requirement of lecithotrophic development, but instead a feature of phylogenetic history.

Phylogeny of echinoid ontogeny

The Cidaroidea have been proposed as the sister group to the other extant echinoids, the Euechinoidea. This position is supported by several features of adult morphology (Jensen, 1981; Smith, 1984a, b), as well as by patterns of gene expression (Raff *et al.,* 1984; Wray and McClay, 1989). Cidaroids are characterized by lantern supports called apophyses, simple ambulacral plates, a narrow upright lantern, and the morphology of the pedicellaria. The euechinoid orders are united by several shared, derived features that distinguish them from cidaroids: lantern supports called auricles, compound ambulacral plates, lanterns with a deep foramen magnum, and details of plate arrangement. Within euechinoids, the Echinothurioida are distinguished by several primitive features absent in other orders, and by unique, derived features, including pseudocompounding of plates.

The absence of a vestibule is a character shared by cidaroids and echinothurioids and might be seen as a derived feature uniting these groups phylogenetically. However, this is probably not the case. Even if the presence of a vestibule and nature of lantern supports alone are considered, parsimony still places echinothurioids and the remaining euechinoids as a monophyletic group (Fig. 10a). The other two possible phylogenetic relationships each require not only more changes, but parallel

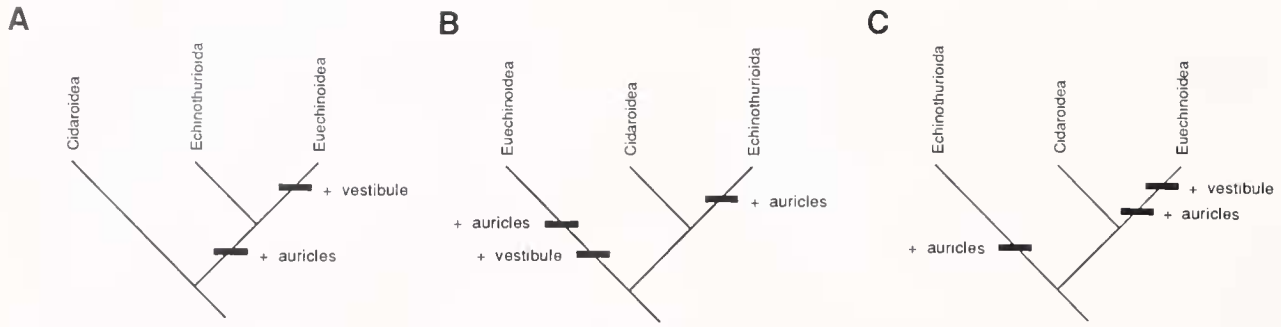

Figure 10. Echinoid phylogeny. The three possible phylogenetic relationships between cidaroids, echinothurioids, and nonechinothurioid euechinoids are shown. The states of two characters are noted in each case: presence of a vestibule during metamorphosis and the nature of lantern supports. Here we assume that the ancestor of post-Paleozoic echinoids lacked a vestibule and possessed cidaroid-type lantern supports. Acquisition of a vestibule and of lantern supports attached to ambulacral rows (euechinoid-type) are indicated "+ vestibule" and "+ auricles," respectively. The most parsimonious phylogenetic hypothesis is represented by cladogram a; cladograms b and c require more changes and parallel evolution of auricles. This hypothesis is supported by additional morphological and molecular data (see text).

evolution of auricles, which are complex morphological characters (Fig. 10b, c). Although they differ in details, the pluteus larvae of cidaroids and euechinoids are sufficiently similar that the pluteus is likely a primitive feature of post-Paleozoic echinoids. Therefore, the extinct common ancestor to cidaroids and euechinoids probably developed via a pluteus larva and lacked a vestibule. A vestibule was probably acquired after echinothurioids split from the remaining euechinoid orders, during early radiation of the euechinoids.

P. parvispinus illustrates the diversity of developmental patterns exhibited by echinoids. The lack of a vestibule, more simple metamorphosis, and the slower rate of development than comparable euechinoids are features common to cidaroids. Other charaeteristics such as large gametes, the absence of micromeres, a wrinkled blastula, altered msp130 expression, and accelerated echinus rudiment formation appear to be associated with the evolution of lecithotrophic development. The ability to differentiate between features attributable to ancestry and those due to the evolution of lecithotrophy should enable us to begin to decipher the molecular and cellular changes necessary to alter developmental mode.

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